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Short title: *V* × *pseudorosulata* in the U.S.

Invasive Eelgrass Hybrid, *Vallisneria* × *pseudorosulata* in the Southeastern United States

Maxwell G. Gebhart[1], Samuel A. Schmid[2], Stephen Turner[3], David Webb[4], Ryan Thum[5], Jens Beets[6], Gray Turnage[7]

[1]Research Associate (0009-0008-2050-5459), Mississippi State University, Starkville, MS, US; [2] Research Associate, Mississippi State University, Starkville, MS, US; [3] Program Manager, Tennessee Valley Authority, Knoxville, TN, US; [4] Senior Program Manager, Tennessee Valley Authority, Knoxville, TN, US; [5] Professor, Montana State University, Bozeman, MT,US ; [6] Research Ecologist, USDA, Davis, California, US; [7] Extension Research Professor, Mississippi State University, Starkville, MS, US

Author for correspondence: Maxwell G Gebhart; Email: mgebhart@gri.msstate.edu

Abstract

Vallisneria × *pseudorosulata* S.Fujii & M.Maki is an invasive aquatic weed that has recently become a major issue within the southeast U.S. *V.* × *pseudorosulata* is a hybrid between two non-native eelgrass species (*Vallisneria spiralis* L. and *Vallisneria denseserrulata* Makino) and has rapidly overtaken waterbodies in Tennessee, Alabama, and Florida. This hybrid can reproduce rapidly through offshoot formation and floating propagules capable of drifting large distances before establishing. *Vallisneria* × *pseudorosulata* has been previously found in Japan and is thought to have been introduced in the U.S. by the aquarium trade or dumping.

Keywords: aquatic macrophyte, aquarium trade, cryptic invader, Vallisneria, submersed

Vallisneria L. is a genus of submersed macrophytes that have often been a major target for phytoremediation and restoration efforts (Cao and Ruan 2015; Korschgen and Green 1988; Les et al. 2008). Often, *V. americana* Michx. is a primary species for restoration efforts in the U.S. due to the natural habitat, forage, and structure it can provide to many ecosystems; however, there has been a steady decline of the species throughout many aquatic systems (Engelhardt et al. 2014). Simultaneously, recent research has determined that there are two native species of *Vallisneria* in the U.S., *V. americana* and *V. neotropicalis* Marie-Victorin (les et al. 2008; Martin and Mort 2023). Thus, most of the current research on *Vallisneria* has focused on how to restore *V. americana* to the landscape and reasons why it may be disappearing. However, there has been a sudden and rapid invasion by a hybrid, *V.* × *pseudorosulata* S.Fujii & M.Maki (= *V. spiralis* × *denseserrulata*) which has received increasing attention in the past 5 years. *Vallisneria* × *pseudorosulata* is thought to be introduced from the aquarium trade as an ornamental *Vallisneria* plant for sale but was later dumped into a waterbody and has rapidly spread throughout the U.S. southeast (Gorham et al. 2021; Wasekura et al. 2016). It is also thought to have been used in native *Vallisneria* restoration under the guise of being a native hybrid (King's Bay Restoration Project). Both parental taxa of *V.* × *pseudorosulata*, *V. spiralis* L. and *V. denseserrulata* Makino, are non-native in the U.S. and originate from the Eurasian continent (Chen et al. 2012; Gorham et al. 2021; Les et al. 2008; Wasekura et al. 2016; Mesterházy et al. 2021). *Vallisneria* × *pseudorosulata* has infested the Tennessee Valley Authority (TVA) system which the population is thought to have established sometime between 2018 and 2019 (Gorham et al. 2021).

The introduction into the TVA system is not an isolated event as *V.* × *pseudorosulata* has been found in multiple systems in Florida, Alabama, Tennessee, and recently Mississippi. Internationally, *V.* × *pseudorosulata* was initially reported in 2016 present in multiple waterbodies throughout Japan (Wasekura et al. 2016). The cross is unlikely to have occurred naturally as both parent species are completely disjunct (Gorham et al. 2021; Wasekura et al. 2016). Since this hybridization, *V.* × *pseudorosulata* has been distributed widely through the aquarium trade under the names *V. spiralis*, *Vallisneria* 'Rock Star', and potentially other common names (Martin and Mort 2023; Wasekura et al. 2016; Padilla and Williams 2004). Gorham et al. confirmed *V.* × *pseudorosulata* primarily in Florida with one site in Alabama which this paper confirms several other sites where *V.* × *pseudorosulata* is found in the southeast U.S. There is little understanding about *V.* × *pseudorosulata* and its ecological interactions with

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other organisms. However, there is anecdotal evidence that *V.* × *pseudorosulata* is rapidly displacing the invasive species *Hydrilla verticillata* L. prompting major concern from resource managers (Gorham et al. 2021; Wetzel 2020). Whether or not *H. verticillata* is being displaced by *V.* × *pseudorosulata*, it is incredibly clear that *V.* × *pseudorosulata* can rapidly become the dominant plant within a waterbody (Gorham et al. 2021). It is currently hypothesized, *V.* × *pseudorosulata* grows as an evergreen perennial and can form large floating mats of propagules, upwards of 10 miles (16 kilometers), which have led to its rapid spread (Plotka 2023; Sapp 2024; Anonymous 2023). The propagules are capable of floating for long stretches and may be why Mississippi was recently invaded from *V.* × *pseudorosulata*. The population of *V.* × *pseudorosulata* found in Mississippi was discovered in June 2024 in Pool E (Prentiss County, nr. Belmont) of the Tennessee-Tombigbee Waterway, a system that is connected to the TVA system, flows into Mobile River, and ultimately drains into the Gulf of Mexico at Mobile Bay (Table 1; *Schmid and Magandy 353 MISSA039771*). This is the first report to document the newest population of *V.* × *pseudorosulata* as well as document the distance of spread from the sampling location Gorham reports in Lake Guntersville.

Morphologically, many *Vallisneria* species are incredibly similar with few diagnostic characteristics; this crypticity equally applies to *V.* × *pseudorosulata* which can impede early detection (Les et al. 2008). *Vallisneria* × *pseudorosulata* looks similar to *V. americana*, growing as a rosette with long, ribbon-like leaves and reduced floral characteristics that can make in-field identification difficult (Les et al. 2008 Martin and Mort 2023). Often, *V.* × *pseudorosulata* can be identified by a bright green lacunal band running up the midvein to the leaf tip; however, this can be difficult to determine on young leaves or if water clarity is an issue (Figure 1d; Figure 2). Another characteristic, and the most diagnostic, is a partially lignified stem that *V.* × *pseudorosulata* will grow just above the soil line (Gebhart obs.; Wasekura et al. 2016). This stem structure was described by Wasekura et al. as a trait that grows on mature plants; but not every *V.* × *pseudorosulata* individual has shown this in the field (Gebhart obs.). This stem can be 1-12 cm in length and typically have small, but apparent internodes with adventitious roots growing from the nodes (Figure 1g and 1h). Currently it is highly recommended that if a *Vallisneria* population should look similar to *V.* × *pseudorosulata*, then genetic testing should be done for confirmation. Genetic testing is highly accurate, and assays have been developed to distinguish *V.* × *pseudorosulata* from *V. americana* and *V. neotropicalis* (Tringali et al. 2023; Martin and Mort

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2023). Should a site contain *V.* × *pseudorosulata*, many *Vallisneria* species typically reproduce via runners that have been observed growing above and below the soil line often with upwards of 10 to 20 daughter plants (Korschgen and Green 1988; Martin and Mort 2023; Mcfarland and Shafer 2008). Anecdotally, these runners have been observed detaching from the parent plant and floating long distances before settling in the sediment again. *Vallisneria* spp. are dioecious, but *V.* × *pseudorosulata* has only been observed with pistillate flowers, leading researchers to hypothesize it does not reproduce sexually (Figure 1i; Gorham et al. 2021; Martin and Mort 2023). Preliminary observations have also shown that *V.* × *pseudorosulata* can handle low to freezing temperatures allowing *V.* × *pseudorosulata* to rapidly establish in the spring (Gebhart obs.). *Vallisneria* × *pseudorosulata* presents not only a unique challenge in the field with identification, but the entire genus of *Vallisneria* has presented multiple complexities for systematists, taxonomists, and evolutionary biologists. Particularly, species like *V. australis* and *V. neotropicalis* are still debated in many circles and often get lumped under the name of a congener (Les et al. 2008; Jacobs and Frank 1997).

The evolutionary and taxonomic history of *Vallisneria* is thought to be another major contributor to the confusion surrounding the invasive *V.* × *pseudorosulata* as well. Lowden (1982) sought to create a taxonomic tree using floral characteristics specifically, thus leading to only two species of *Vallisneria*, *V. spiralis* and *V. americana*. However, the genus was re-evaluated in both 2008 and 2023 which both determined genetically there are most likely 12 to 16 species scattered throughout the world (Les et al. 2008; Martin and Mort 2023). Both in 2008 and 2023, arguments were made for the distinction of two native *Vallisneria* species in North America, *V. americana* and *V. neotropicalis* (Les et al. 2008; Martin and Mort 2023; Marie-Victorin 1943). *V. americana* is well understood and has presence throughout the U.S. however, *V. neotropicalis* has little knowledge about its range and biology. Both native species have been observed co-occurring with *V.* × *pseudorosulata* which, again, can make *in situ* identification difficult (Figure 2 and 3). These co-occurrences have been genetically confirmed through sequencing ITS sequencing with the sampled populations found in Figure 3. We recommend that systems connected to known populations of *V.* × *pseudorosulata* be monitored for invasion, and when new populations of *Vallisneria* are observed they should be examined for an elongated stem and ideally, genetically confirmed. Current invasions have taken over impressively large

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areas of the TVA system and more recently in Mississippi suggesting further spread throughout the Tennessee River system.

The current observations of *V.* × *pseudorosulata* tied with how little information is present raises great concern for further spread throughout the southeastern U.S. *V.* × *pseudorosulata* was first recorded in the U.S. recently; however, the rate at which invasion and spread is of major concern. Gorham et al. previously reported only one geographic point in Lake Guntersville which *V.* × *pseudorosulata* is found, however, our report shows both upstream and downstream spread. Our report is also the first to have visual representations of the structures that make *V.* × *pseudorosulata* unique for identification and describe problems within field identification. There is a dearth of cohesive research for many of the species in *Vallisneria*, including *V.* × *pseudorosulata*, therefore, future studies should strive towards creating comprehensive information focused on ecological impacts, biological traits, and management of *V.* × *pseudorosulata*.

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Competing Interests

The authors declare none.

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Table 1. Collection, date, and location details of *Vallisneria* × *psuedorosulata* collected in the United States in 2023 and 2024. All specimens have been submitted and digitized in the Mississippi State University herbarium (MISSA).

Primary collector	Collection number	Catalog number	Date	State	Latitude (°)	Longitude (°)
S. Turner	1	*MISSA039774*	Sep 2023	Alabama	34.6421	-85.9696
S. Turner	2	*MISSA039775*	Sep 2023	Tennessee	35.0634	-85.5323
S. Turner	3	*MISSA039769*	Sep 2023	Alabama	34.5601	-86.8517
S. Turner	4	*MISSA039770*	Sep 2023	Tennessee	35.1561	-85.1559
S.A. Schmid	353	*MISSA039771*	Jun 24, 2024	Mississippi	34.4758	-88.3346

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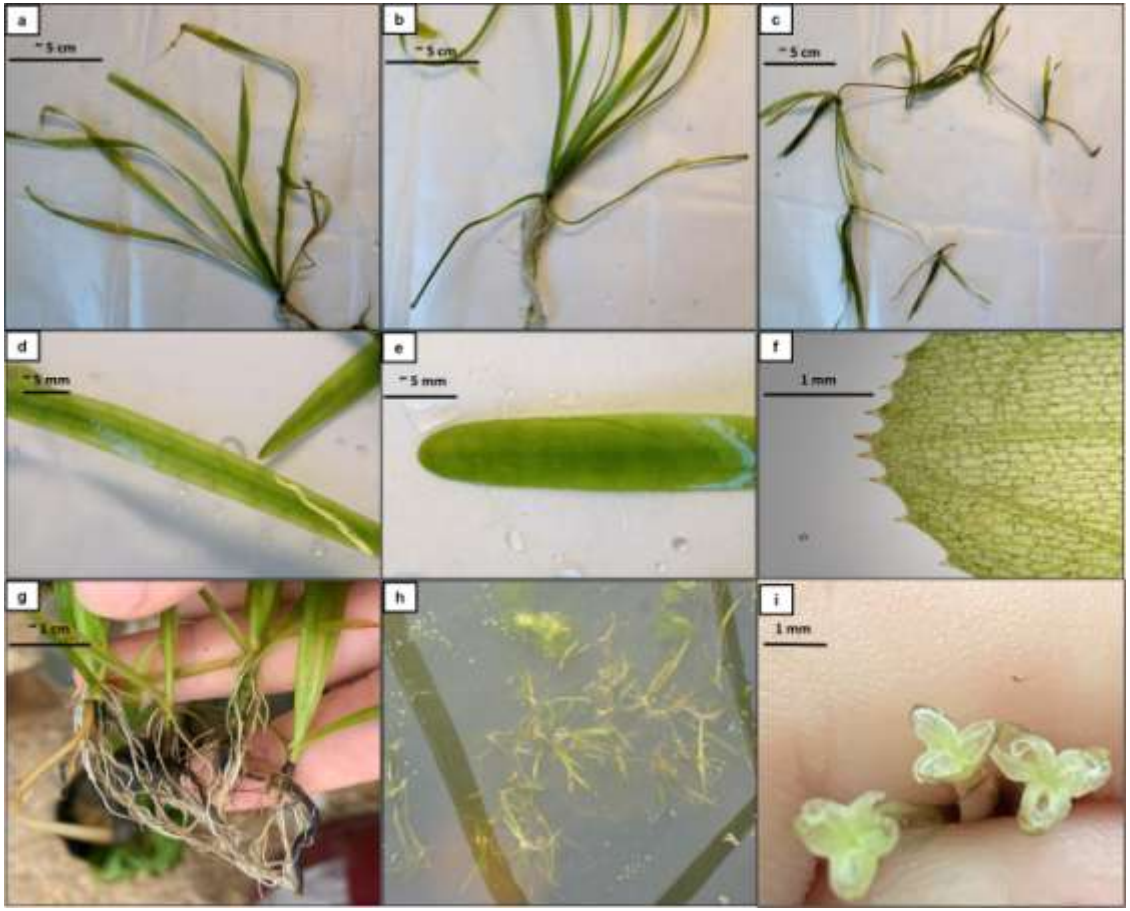

Figure 1. a) The typical growth form of *V.* × *pseudorosulata* as a rosette with long, thin leaves; b) asexual reproduction usually takes form of stolons that extend from the rosette base; c) when established, stolons with many small ramets can extend more than 30 cm; d) *V.* × *pseudorosulata* leaves are denoted by bright green lacunal band that follows the midvein; e) leaf tips for *V.* × *pseudorosulata* are obtusely angled and appear rounded; f) serrations are small and dense along leaf margins; g) stem formation by *V.* × *pseudorosulata* during experimentation in mesocosms; h) chains of ramets can form in both still and moving water which can allow for new establishment once the ramets sink.; and i) pistillate flowers of *V.* × *pseudorosulata* which are the only found flower sex currently.

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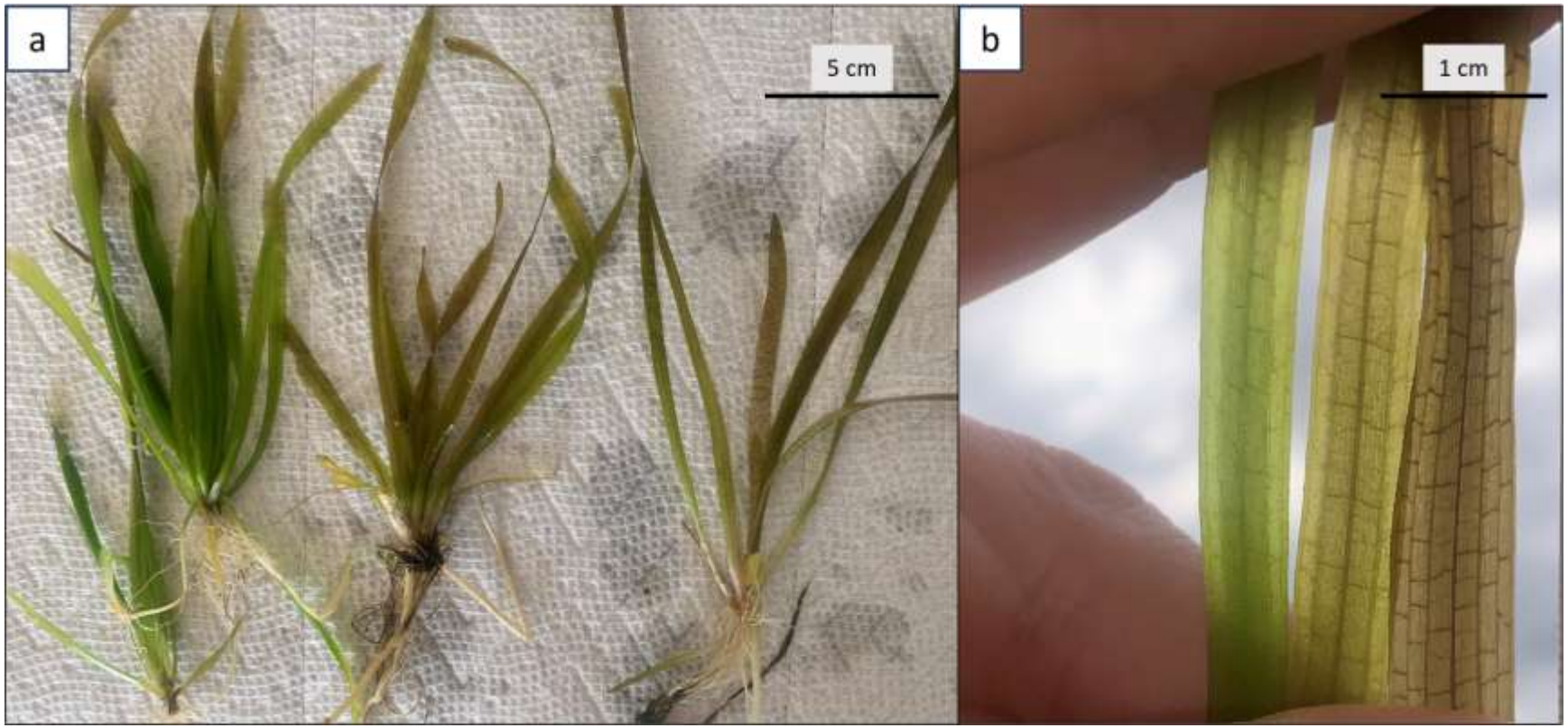

Figure 2: a) A comparison between *V.* × *pseudorosulata* (left), *V. americana* (center), and *V. neotropicalis* (right). b) A comparison of the leaf colorations between *V.* × *pseudorosulata* (left), *V. americana* (center), and *V. neotropicalis* (right). Both *V. americana* and *V. neotropicalis* leaves can have different colorations based on the growing depth, but may be typically darker than *V.* × *pseudorosulata*.

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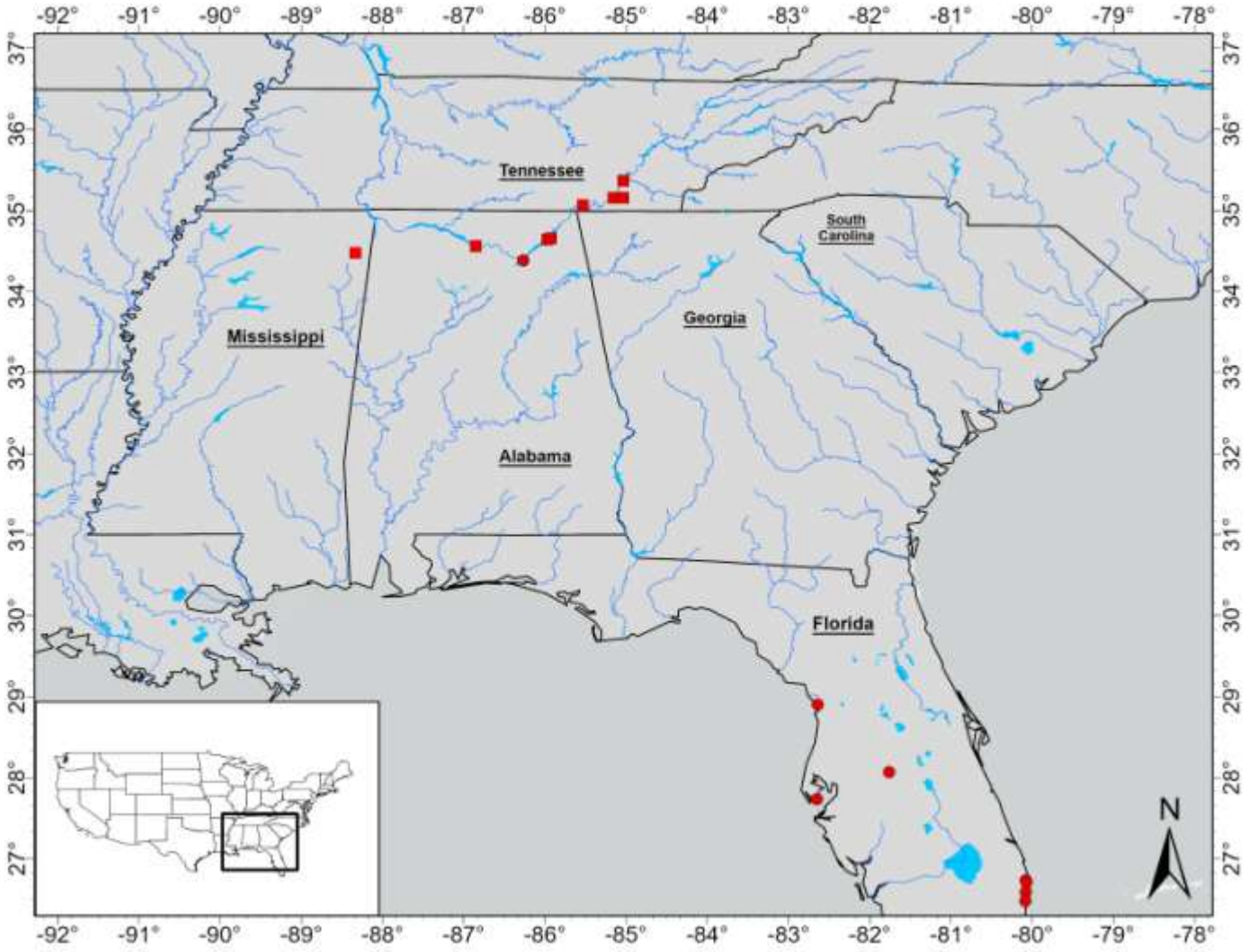

Figure 3. A map displaying known locations of *V.* × *pseudorosulata* in the southeastern U.S. The circles represent approximate sampling locations from Gorham et al. 2021 and the squares represent the population extent being reported.

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